SHORT COMMUNICATION

Epidemiology of *Pasteurella multocida* in a Farrow-to-Finish Swine Herd

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ABSTRACT

Thirty-eight clinical isolates of *Pasteurella multocida*, recovered from a continuous flow, farrow-to-finish swine herd, were characterized by capsular serotyping and restriction endonuclease analysis (REA) in order to study the epidemiology of *P. multocida* pneumonia. Twenty-three of the 38 isolates obtained in the study belonged to serotype A. They displayed three REA patterns after digestion with *Hpa*II, of which one designated A-3 represented 70% of the samples. The remaining 15 isolates were serotype D. Four different REA patterns were observed in the type D isolates. The REA type D-1 was most prevalent and accounted for 47% of the serotype D isolates. All serotype A isolates were nontoxigenic, whereas five (33%) of the serotype D isolates were toxigenic. Vertical transmission of *P. multocida* could not be demonstrated, and was probably not a major route of infection. The results of this study suggest that strains of *P. multocida* virulent for pigs exist and cause swine pneumonic pasteurellosis in continuous flow herds by horizontal transmission.

RÉSUMÉ

Dans le but d'étudier l'épidémiologie de la pneumonie à *Pasteurella multocida*, 38 souches, isolées d'un élevage en continu de type naisseur-finisseur, ont été caractérisées par typage du matériel capsulaire et par analyse de patrons génomiques obtenus à l'aide d'une endonucléase de restriction. Vingt-trois des 38 souches appartenaient au sérotype A. Elles démontraient, après digestion de l'ADN génomique avec l'endonucléase de restriction *Hpa*II, 3 patrons génomiques dont un, appelé A-3, représentait 70 % des échantillons. Les 15 autres souches appartenaient au sérotype D et ont démontré 4 patrons génomiques. Le patron D-1 était le plus prévalent et représentait 47 % des souches de sérotype D. Toutes les souches de sérotype A étaient non-toxinogènes, tandis que cinq (33 %) des souches de sérotype D étaient toxinogènes. La transmission verticale de *P. multocida* n'a pu être démontrée, et ne représente probablement pas la voie majeure de l'infection. Les résultats de cette étude suggèrent que les souches de *P. multocida* virulentes pour le porc sont présentes et peuvent causer une pneumonie dans les élevages de type naisseur-finisseur via une transmission horizontale. (*Traduit par Dr Mario Jacques*)

Swine pneumonic pasteurellosis is an important and costly disease in the swine industry. *Pasteurella multocida* serotype A strains play a central role in the development of disease. In the United States, the majority of isolates recovered from swine lungs associated with pneumonia have been serotype A (1). At present, the most commonly used method for the differentiation of *P. multocida* strains is capsular and somatic serotyping (2–4). This technique can only determine which serological strain of *P. multocida* is involved. It cannot differentiate individual strains associated with disease within a given serotype, nor identify virulent strains in swine populations.

Recently, Zhao *et al* (5) demonstrated that chromosomal restriction endonuclease analysis (REA) and ribotyping were useful epidemiological methods to study transmission patterns of *P. multocida* and to evaluate the relationship between prevalent strains and other strains present in closed, all-in all-out swine herds. The objective of this study was to use REA to differentiate isolates of *P. multocida* recovered from a continuous flow, farrow-to-finish swine herd during a natural outbreak of pneumonic pasteurellosis. The results presented here showed that strains could be distinguished within serotypes using REA. Furthermore, the data indicate that the spread of the organism within the herd was by horizontal rather than vertical transmission.

A 150-sow, continuous farrow-to-finish operation in southern Minnesota was selected for study. It was a one-site production farm consisting of

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TABLE I. Serotypes and restriction endonuclease analysis profiles of *P. multocida* isolates from pigs

Serotype	REA profile	No. of isolates from			
		Sow	Nursery	Grow-Finish	Lung
A	A-1	1	2	2	0
A	A-2	0	0	0	2
A	A-3	0	0	7	9
D	D-1	0	0	6	1
D	D-2	0	1	3	0
D	D-3	0	1	1	0
D	D-4	0	0	2	0

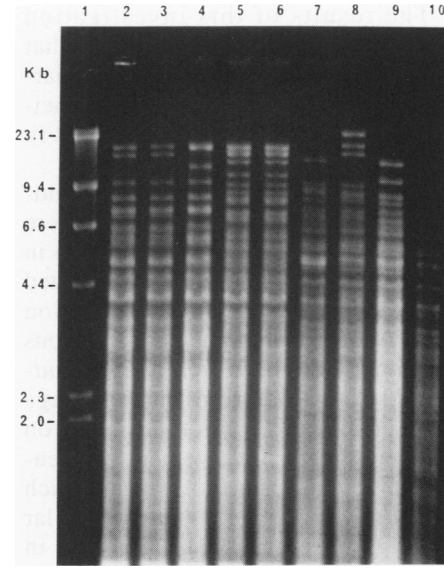

Fig. 1. Restriction fragment patterns of the REA types of *P. multocida* strains isolated from pigs in a farrow-to-finish herd. Samples were digested with *Hpa*II and electrophoresed in 0.55% agarose as described in Materials and Methods. Lanes: 1, lambda DNA digested with *Hin*dIII; 2, strain S3 (REA type A-1); 3, strain N16 (A-1); 4, strain L27 (A-2); 5, strain L2 (A-3); 6, strain F12 (A-3); 7, strain F17 (D-1); 8, strain F8 (D-2); 9, strain F3 (D-3) and 10, strain F4 (D-4). Kb, kilobases.

penned gestation, farrowing and nursery rooms, and a grow-finish barn. There were two farrowing rooms. One was newly remodeled with ten farrowing crates. The other contained penned farrowing facilities. The nursery room had ten pens with mechanical ventilation. Piglets were weaned at four weeks of age into the nursery room. The grow-finish barn was naturally ventilated and consisted of ten pens holding about 45 to 50 pigs per pen. Finishing pigs were selected for market when they reached about 250 pounds of body weight.

Ten farrowing sows of various parities; three piglets from the litter of each sow; 20 nursery pigs 8–12 weeks of age, and 30 grow-finish pigs were randomly sampled from the oropharynx with sterile cotton swabs (Culturette; Becton Dickinson Microbiology Systems, Cockeysville, Maryland). Thirty-eight lung samples from these finishing pigs were also collected at slaughter for *Pasteurella* isolation one day after the on-farm screening.

Isolation of *P. multocida* from oropharyngeal swabs was achieved by a modified mouse inoculation technique as described previously (6). The procedures for characterization of *P. multocida* isolates, toxin detection, DNA extraction and restriction endonuclease analysis have been previously described (5).

Differences between percent pneumonic tissue involvement in lungs with positive *Pasteurella* isolation and lungs without isolation were analyzed by a rank-sum, two sample (Mann-Whitney) test.

Affected animals showed classical symptoms of respiratory disturbance. Clinical signs of pneumonia characterized by coughing, labored breathing and atrophic rhinitis with snout distortion were detected in grow-finish pigs. Ninety-eight percent of lungs examined at slaughter had pneumonic lesions. The major macroscopic lesions were found in the anteroventral lobes of the lungs and were purple to grey in color. Affected regions were firm and appeared atelectatic. The common microscopic lesions were acute purulent pneumonia indicative of pasteurellosis and peribronchiolar lymphocytic cuffing most likely indicating *Mycoplasma hyopneumoniae* infections.

Only one serotype A isolate was recovered from the oropharyngeal swabs of sows and none from their litter piglets. Four and 21 isolates of *P. multocida* were recovered from nursery and grow-finish pigs, respectively. In addition, 12 isolates (including one serotype D) were obtained from 12 pneumonic lungs. The serotype distribution of these isolates is shown in Table I. Only 5 (33%) of the 15 serotype D *P. multocida* isolates obtained from oropharyngeal swabs were toxigenic. All 23 serotype A isolates were nontoxigenic.

The percent of pneumonic lung tissue found in the 12 lungs with positive *Pasteurella* isolation ranged from 6% to 46%, with a mean percent pneumonia of 30%. In contrast, the percent of pneumonic lesions of 25 pneumonic lungs with negative isolation ranged from 2 to 36%, with a mean pneumonic score of 12%. The percent pneumonic tissue involvement in lungs with positive *Pasteurella* isolation was significantly higher than those pneumonic lungs with negative isolation ($p < 0.01$). Only one apparently normal lung was observed and it was negative for *P. multocida*.

All *P. multocida* isolates were analyzed for their restriction endonuclease patterns by DNA digestion with *Hpa*II. Three different REA patterns, designated as REA types A-1, A-2 and A-3, were observed from the 23 serotype A isolates (Table I). A representative example of these patterns is presented in Fig. 1. Restriction endonuclease analysis type A-3 (Fig. 1, lanes 5 and 6) appeared to be identical to the REA type 4 previously found in a closed herd (5). This strain was predominant and represented 70% of the isolates collected. The REA types A-1 (Fig. 1, lanes 2 and 3) and A-2 (Fig. 1, lane 4) accounted for 22% and 9% of the samples, respectively. Among the 15 serotype D isolates of *P. multocida*, four different REA patterns, designated as REA types D-1 through D-4 (Fig. 1, lanes 7 through 10, respectively) were observed. The REA type D-1 was most prevalent and represented 47% of serotype D isolates. The second most common REA type, D-2, accounted for 27%. The majority of these two REA types were isolated from grow-finish pigs (Table I). The only serotype D isolate recovered from a pneumonic lung belonged to REA type D-1.

The results of this investigation support our earlier observations that virulent strains of *P. multocida* apparently exist for pigs and may be associated with pneumonic pasteurellosis in swine populations (5).

The clinical and pathological findings from pigs in this study herd were consistent with enzootic pneumonia in swine and usually have been associated with *P. multocida* secondary infection (7). The presence of pneumonic lesions in animals correlated well with *P. multocida* isolation from lungs. Lungs with positive *P. multocida* isolation also had greater involvement of pneumonic lesions than those from which *P. multocida* was not isolated. Similar findings were observed previously in swine herds reared under all-in all-out management (5).

In the current study, serotypes A and D were recovered from the oropharynx of live pigs on the farm in approximately equivalent numbers. The relatively high isolation rate of *P. multocida* may be due to larger numbers of organisms colonizing the tonsils of pigs reared conventionally compared to those in high health status herds. It is also probable that the mouse inoculation technique enhanced the isolation of *P. multocida* from pigs, as suggested by de Jong *et al* (8).

Previous research has shown that both serotype A and D strains of *P. multocida* can colonize the tonsils of swine (9). *Pasteurella multocida* serotype A strains were readily recovered from both pneumonic lungs and tonsils of pigs, while almost all serotype D isolates were obtained from tonsils (see Table I). Serotype A strains of *P. multocida* are not easily phagocytosed by alveolar macrophages *in vitro* (10), presumably because they have a large hyaluronic acid capsule. They may thus be able to colonize and multiply in the lung and cause pneumonia. However, healthy pigs are capable of readily clearing type A strains from the lung. This suggests that the ability of these strains to cause pneumonia may be associated with factors other than phagocytosis. Conversely, infectious agents that allow *P. multocida* to colonize the lung may do so by interfering with normal clearance mechanisms. Serotype D strains can colonize the upper respiratory tract of pigs (9). However, they seem unable to survive in the lung and cause significant damage to lung tissues. This may explain why serotype D strains were less commonly found in the lung, and are probably not an important causative factor for pneumonia.

Restriction endonuclease analysis revealed three different types within the 23 serotype A isolates, and four REA types in the 15 serotype D *P. multocida* isolates obtained from the farrow-to-finish herd. Although various REA types were observed, REA type A-3 was the predominant strain, suggesting an association with pneumonia. Interestingly, this strain was identical to a previously isolated strain, which was shown to be of low prevalence in a herd from a different region in the state (5). This suggests that *P. multocida* strains with the same REA type have different degrees of virulence under different management conditions.

The epidemiology of *P. multocida* appeared to involve horizontal transmission, since no *P. multocida* could be recovered from suckling piglets. Although strain A-1 was isolated from animals in different production stages, it did not appear to be associated with disease. Conversely, strain A-3, the most common lung isolate, was not found in the breeding herd or the nurseries. The source of infection was most likely from other pigs, suggesting that *Pasteurella* carrier animals in the herd were an important source of infection. Disease transmission within this herd was probably by direct nose to nose contact between pigs. In addition, animals were moved and mixed with other groups from time to time during different stages of production, making the spread of disease more likely.

It was shown previously (5) that in closed swine populations, a single strain of *P. multocida* predominates and appears to be associated with pneumonic pasteurellosis. The present study confirmed this observation. In addition, restriction endonuclease analysis was able to provide information regarding the relatedness of isolates from different growth stages of pigs that could not be differentiated by serotyping or other phenotyping methods. It is concluded that REA in combination with capsular serotyping is an appropriate tool that can be used for further differentiation of *P. multocida* strains.

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